GUIDELINE: 83-3

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DATA EVALUATION RECORD

Study Type: Teratology - Developmental Toxicity
Species: rat
Guideline: 83-3

EPA Identification No.s: EPA MRID No. 413770-02
EPA ID No. 066501
EPA Record No. S382263
Caswell No. 031
HED Project No. 1-0039

Test Material: Phosphine 1% in Nitrogen gas

Synonyms: PH_3

Sponsor: Metal Phosphide Task Force
DEGESCH America, Inc.
Weyers Cave Virginia

Study Number(s): 89-3413

Testing Facility: Bio/Dynamics, Inc.
East Millstone, N.J.

Title of Report: An Inhalation Developmental Toxicity Study of Phosphine in Rats

Author(s): Raymond E. Schroeder

Report Issued: December 5, 1989

Conclusions: Under the conditions of this study, the maternal NOEL is 5 ppm and the maternal LEL is 7.5 ppm based on the high incidence of maternal deaths. The reproductive NOEL is 5 ppm and the developmental NOEL is 5 ppm. Doses tested in this study were 0, 0.03, 0.30, 3.0, 5.0, and 7.5 ppm.
Core Classification: Minimum. The study satisfies the requirements for developmental toxicity as set forth in the Subdivision F Guidelines.

A. Materials:

Test Compound: Phosphine
Description: Colorless gas with unpleasant odor
Lot Nos.: N-429433, H-70377, N-299593, N-411001

Vehicle(s): 1% Nitrogen gas

Test Animal(s): Species: rat
Strain: CD derived Sprague Dawley
Source: Charles River
Portage, Michigan
Age: Approx. 10 weeks at start of study
Weight: 186 - 281 grams

B. Study Design :

This study was designed to assess the developmental toxicity potential of Phosphine when administered by the inhalation route to female rats on gestation days 6 through 15, inclusive.

Mating

Following a one month acclimation period, female rats were mated with untreated male rats at a ratio of 1M:1F. Matings took place at night and on the morning following cohabitation, the females were examined for the presence of sperm and/or a vaginal plug.

Matings and exposures to phosphine were not conducted on the same day for all animals. Mating was conducted over a period of 19 nights and following each night of mating, females were sorted into groups on the following morning.

Rooms were maintained at temperatures of 67 to 80° F and relative humidity of 30 to 70%.

Group Arrangement:

Test Group	Dose Level (ppm)	Number Assigned
Control	0	24
II	0.03	27[a]
III	0.30	24
IV	3.0	24
V	5.0	24
VI	7.5	19[b]

a= three animals sacrificed on day 2 of exposure were replaced with 3 other females.

b = due to excessive mortality, this group was terminated early in the study.

Dosing

All animals were exposed to levels of phosphine as designated by group number on days 6 through 15 of gestation. Phosphine was administered as a vapor into the breathing zone in 6 m^3 stainless steel and glass chambers. Animals were exposed for six hours each day and remained in the chamber 30 minutes following each exposure to allow the vapors to clear before removing the test animals. Control animals were exposed to room air only during the 6 hour exposure period.

Experimental Chambers

Harford glass and stainless steel exposure chambers with a total volume of 6000 liters were used. The airflow rate, time for air exchange and 99% equilibrium time were measured for each group of animals. Phosphine was delivered by a stainless steel regulator which was connected to a 1/4 swaglock union cross where the test substance was carried to 5 exposure chambers via stainless steel tubing. The test substance was delivered to the top turret of the exposure chamber.

Gas chromatography was used to detect phosphine exposure levels and the nominal concentration of phosphine was determined by dividing the total volume of phosphine metered into the tube by the total volume of air passing through the chamber. Temperature, relative humidity and chamber volumetric air flow were measured every 1/2 hour. (See Table V for chamber monitoring results).

Observations

Maternal animals were observed twice daily for mortality and for clinical signs of toxicity. Physical exams were conducted on days 0, days 6-15 and day 20 of gestation. Body weights were recorded on days 0, 6, 10, 12, 16 and 20 post-coitum. On day 20, actual and corrected body weights were recorded. Food consumption was determined at 4 intervals from day 0 thru day 20. On day 20 post-coitum, animals were sacrificed by exsanguination while under light ether anesthesia.

The ovaries and uterus were removed from each animal and the following parameters were assessed:

Number of corpora lutea
Number of implantations
Number of live fetuses
Number of early resorptions
Number of late resorptions
Number of dead fetuses

Non-pregnant females were immersed in 10% ammonium sulphide to reveal the number of implantation sites. Females with foci in the uterus that were observed after special staining, were considered as having been pregnant.

Fetuses were identified, sexed and subjected to a gross examination for external malformations/variations. One half of the fetuses were evaluated for visceral abnormalties; the tissues were fixed in Bouin's solution. The remaining half were subjected to a skeletal evaluation after being stained for ossified structures using Alizarin red.

Late resorptions were examined grossly for external malformations. Those with malformations were saved in 10% neutral buffered formalin.

Historical control data were not provided to allow comparison with concurrent controls.

Statistical analysis
Statistics were conducted on data and comparisons were made between control and treated groups. Statistics included Bartlett's test for variance, parametric (ANOVA, Dunnett's test) and non-parametric (Kruskall-Wallis) procedures. Tests for trend included standard regression techniques (parametric) and Jonckheere's test(non-parametric. Arc sine transformation was used on all ratios prior to analysis. $P \leq 0.05$ and $p \leq 0.01$ were determined.

Compliance
A signed Quality Assurance Statement dated 10/24/89 was provided. A statement of compliance with GLPs was also provided.

C. Results

Maternal Toxicity

Mortality
No mortality was reported in groups receiving phosphide up to 5 ppm. At 7.5 ppm, the first 14 females assigned to the group died during the exposure period. The number of exposures that animals in this group received prior to death ranged from 3 to 10. The remaining five animals that had not been exposed to phosphide were sacrificed and the entire 7.5 ppm group was removed from the study.

Clinical Observations
No clinical signs of toxicity were present at doses lower than 7.5 ppm.

Body Weight
Mean body weights and body weight gains were comparable between groups. In terms of absolute weight gains during the study, the treated animals gained more weight than controls. Animals in the 7.5 ppm group were not included in the data analysis. (See Table I, derived from data provided by the sponsor).

Food Consumption:
Food consumption was comparable for all groups for the period prior to dosing and for the period from day 6 to 16. During the post treatment interval, animals in the 5.0 ppm group had significantly higher food consumption. This finding was not believed to be related to the adminstration of phosphine. (See Table II).

Gross Pathology:
Most of the lesions observed in this study were in the 14 animals that were in the 7.5 ppm group. The lungs and the liver were the primary organs with pathology. Discoloration of both of these organs was reported (6/14 lungs and 3/14 livers).

Discolored lungs were observed in 1/24, 2/27, 1/24 and 1/24 animals that received 0, 0.03, 0.3 and 5 ppm , respectively. Dilated renal pelvises were reported in all groups of animals receiving phosphine, but without a dose related increase in the frequency. Emphysema was reported in one animal which received 3 ppm. Lymph node enlargement was reported at the two lowest doses of phosphine but was not present at 3 and 5 ppm. Hair loss was reported in one control animal, in one animal receiving 0.03 ppm and in two animals receiving 5 ppm.

None of these findings were believed to be related to the adminstration of phosphine.

REPRODUCTIVE EFFECTS
In the low dose group (0.03 ppm) the mean number of resorptions and the mean number of litters with resorptions were statistically significantly higher than controls. However, this observation was not made in groups receiving higher levels of phosphine (no data were provided for the animals receiving 7.5 ppm). This increase in the mean number of resorptions is not believed to be compound related since similar findings were not present in animals receiving higher levels of the compound. (See Table III, derived from data provided by the sponsor).

FETAL EFFECTS
No compound related observations were made with regard to the external, visceral or skeletal malformations in fetuses from dams exposed to phosphine. External malformations included one fetus with a filamentous tail in the 0.03 ppm group. In the 0.3 ppm group, one fetus had both micrognathia and a rudimentary tongue; in the 5 ppm group, one fetus was both edematous and had a curly tail. No skeletal malformations were observed in control and 3 ppm dose groups.

Visceral variations in the control group consisted of tortuous

ureters in four fetuses (involving three litters). This abnormality was also present at all doses above 0.03 ppm, but with a lesser frequency than in controls (2 fetuses /2 litters at 0.3 and 3.0 ppm and 2 fetuses /1 litter at 5 ppm).

Malformations included microphthalmia which was observed in 1 fetus in both the 0.03 group and the 3.0 ppm group. Cleft palate was present in the same fetus at 3 ppm . Folded retinas were present in one animal in the lowest and one animal in the highest dose groups. One fetus in the high dose group had abnormalities that were limited to the cardiovascular system and included persistent truncus arteriosus, absence of the ductus arteriosus and the presence of a ventricular - septal defect.

No skeletal malformations were present in the high dose or control fetuses. In the low dose group, one fetus had a variety of skeletal malformations (absent lumbar, sacral and caudal vetebrae and a filamentous tail) At 0.3 ppm, one fetus had a short, thickened mandible and a misshapened palatine process and another fetus from a different litter had a cervical rib. At 3 ppm, skeletal malformations included presence of a cervical rib, branched cervical transverse processes, absent thoracic transverse processes, decrease in the number of thoracic vertebrae, fused ribs and the presence of 5 lumbar vertebrae.

Skeletal variations were present in all groups at similar frequencies and were not associated with the adminstration of the test compound.

Because of the low incidence of malformations and the low number of litters affected, the findings are not believed to be associated with the administration of phosphine. (See Table IV for external, visceral and skeletal malformations).

Analytical data

Table I: Mean Body Weight Gains (grams)[a]

Group:	Prior to Dosing Period	Dosing Period	Post Dosing Period	Entire Gest'n Period	Corrected Dosing Period	Entire Study
Control	29	44	53	126	-24.5	48.5
0.03	29	45	51	125	-22.3	51.7
0.30	25	45	54	124	-19.1	50.9
3.00	28	43	56	127	-18.8	52.2
5.00	31	43	58	132	-19.5	54.5

a = data extracted from study report.

Food Consumption

Table II: Mean Food Consumption Data (g/kg/day)[a]

Group:	Prior to Dosing Period	Dosing Period*	Post-Dosing Period	Entire Gestation Period
Control	84	152	83	319
0.03	86	151	86	323
0.30	81	155	85	321
3.00	87	150	86	323
5.00	89	153	91**	333

a = data taken from report submitted by sponsor

* = Dosing period covers days 6-15; however, food consumption measured during the interval which covered days 10-16

** = $p \leq 0.05$

Cesarean section Observations

Table III: Cesarean Section observations[a]

Dose:	Control	0.03	0.30	3.0	5.0
#Animals Assigned	24	27	24	24	24
#Animals Mated/Inseminated	24	27	24	24	24
Pregnancy Rate (%)	91.7	87.5	100	95.8	95.8
Maternal Wastage					
#Died (Total)	0	0	0	0	0
#Died/pregnant	0	0	0	0	0
#Non pregnant	2	3	0	1	1
#Aborted	0	0	0	0	0
#Premature Delivery	0	0	0	0	0
Total Corpora Lutea	353	342	383	365	370
Corpora Lutea/dam	16.0	16.3	16.0	15.9	16.1
Total Implantation	339	329	352	346	355
Implantations/Dam	15.4	15.7	14.7	15.0	15.4
Total Live Fetuses	327	296	335	326	338
Live Fetuses/Dam	14.9	14.1	14.0	14.2	14.7
Total Resorptions	12	33*	17	20	17
Resorptions/Dam	0.5	1.3*	0.7	0.9	0.7
# Litters w/resorptns(%)	8(36)	16(76)	9(38)	14(61)	10(44)
Total Dead Fetuses	0	0	0	0	0
Dead Fetuses/Dam					
Mean Fetal Weight (gm)	3.24	3.18	3.26	3.28	3.22
Preimplantation Loss(%)	4.0	3.9	8.1	5.2	4.1
Postimplantation Loss(%)	3.5	10.0	4.8	5.7	4.8
Male: female ratio	1.0	0.9	1.1	1.1	1.0

[a] = Data extracted from (study or report number and tables or appendices used)

* $p \leq 0.05$

Developmental Toxicity

Table IV: External Examinations[a]

Observations	DOSE 0	0.03	0.30	3.0	5.0
#pups(litters) exmnd	327(22)	296(21)	335(24)	326(23)	338(23)
#pups(litters) affctd	0	1(1)	1(1)	0	1(1)
fetal (litter) incidence					
edematous	0	0	0	0	1(1)
filamentous tail	0	1(1)	0	0	0
micrognathia	0	0	1(1)	0	0
rudimentary tongue	0	0	1(1)	0	0
curly tail	0	0	0	0	1(1)

Table IV: Visceral Examinations

Observations	DOSE 0	0.03	0.30	3.0	5.0
#pups(litters) exmnd	161(21)	151(21)	174(24)	167(23)	175(23)
#pups(litters) affctd	4(3)	2(2)	1(1)	1(1)	2(2)
fetal (litter) incidence					
Cleft palate	0	0	0	1(1)	0
microphthalia	0	1(1)	0	1(1)	0
folded retina	0	1(1)	0	0	1(1)
distended 3rd ventr.	0	1(1)	0	0	0
Cardiovascular defects (truncus arteriosus, absent ductus arteriosus ventricular-septal defect)	0	0	0	0	1(1)

Table IV: Skeletal Examinations

Observations	DOSE 0	0.03	0.30	3.0	5.0
#pups(litters) exmnd	158(22)	146(21)	163(24)	159(23)	163(23)
#pups(litters) affected	0	1(1)	2(2)	3(3)	0
fetal (litter) incidence					
short mandible	0	0	1(1)	0	0
misshapen basisphenoid	0	0	1(1)	0	0
misshapen palatine proc.	0	0	1(1)	0	0
cervical rib	0	0	1(1)	1(1)	0
branched cerv.trans proc.	0	0	0	1(1)	0
abs. thor. trans . proc.	0	0	0	1(1)	0
decr. in # of thoracic	0	0	0	1(1)	0
5 lumbar vetebra	0	0	0	1(1)	0
abs. lumbar vert.	0	1(1)	0	0	0
abs. sacral vert.	0	1(1)	0	0	0
abs.caudal vert.	0	1(1)	0	0	0
fused ribs	0	0	0	1(1)	0

a = data taken from study report.

D. Discussion/Conclusion

Based on the results from this study, phosphine, when delivered via the inhalation route was not associated with maternal, reproductive or developmental toxicity at doses up to 5 ppm. At 7.5 ppm, a high incidence of maternal deaths occurred and this group had to be removed from the study. No analysis was made with regard to the effect that phosphine at 7.5 ppm had on the developmental and reproductive parameters.

All of the reported malformations lack a dose related increase in the frequency of their occurence and are at such a low number that they are not considered to be related to the administration of phosphine.

Maternal, developmental and reproductive NOELs = 5 ppm. The maternal LEL is 7.5 ppm, based on the deaths reported at that dose level.

E. Core Classification: Minimum